Differences in susceptibility of white grub species to entomopathogenic nematodes: the relative contribution of symbiotic bacteria and nematodes

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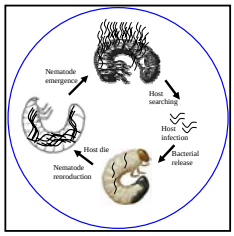
Abstract: As susceptibility of white grub species to entomopathogenic nematodes differs, we compared the virulence of *Photorhabdus temperata* and *Xenorhabdus koppenhoferi*, the symbionts of nematodes *Heterorhabditis bacteriophora* and *Steinernema scarabaei*, respectively, to three white grub species *Popillia japonica*, *Rhizotrogus majalis*, and *Cyclocephala borealis*. Both bacteria were virulent to all three grub species even at as low as 2 cells /grub. However, the median lethal dose at 48 h post injection and median lethal time at 20 cells /grub showed that *P. temperata* was more virulent than *X. koppenhoferi* to *C. borealis*. There were no differences in virulence of two bacteria against *P. japonica* and *R. majalis*. Although *H. bacteriophora* carrying *P. temperata* is less pathogenic than *S. scarabaei* carrying *X. koppenhoferi* to *R. majalis*, *P. temperata* grew faster than *X. koppenhoferi* both *in vitro* and *in vivo*. We then tested the pathogenicity of oral and hemolymph introduced *H. bacteriophora* to *R. majalis* to determine whether nematodes are able to successfully vector the bacteria into the hemolymph. Hemolymph injected *H. bacteriophora* were pathogenic to *R. majalis* indicating successful bacterial release, but orally introduced *H. bacteriophora* were not. Dissection of grubs confirmed that orally introduced *H. bacteriophora* were unable to penetrate into the hemolymph through the gut wall. Therefore, we conclude that the low susceptibility of *R. majalis* to *H. bacteriophora* is not due to the symbiotic bacteria, but is due to the nematode's poor ability to penetrate through the gut wall to vector the bacteria into the hemolymph.

INTRODUCTION

❖ The entomopathogenic nematodes, *Steinernema scarabaei* and *Heterorhabditis bacteriophora*, carrying symbiotic bacteria, *Xenorhabdus koppenhoferi* and *Photorhabdus temperata*, respectively, have potential for biological control of white grubs (Coleoptera: Scarabaeidae).

❖ However, white grub susceptibility differs to nematode species. *H. bacteriophora* is much less pathogenic than *S. scarabaei* to European chafer *Rhizotrogus majalis*, but the reverse is true for the Northern masked chafer *Cyclocephala borealis* (Grewal et al., 2002; Koppenhofer et al., 2007)

❖ The life cycle of nematodes was initiated by the stage of infective juveniles that carrying the bacteria find and enter into the insect host, and release the bacteria into the hemolymph (Fig. 1).



❖ As both bacteria and nematodes are important in establishing a successful infection (Fig. 1), we determined the relative contribution of the bacteria and nematodes in pathogenicity of *H. bacteriophora* and *S. scarabaei* to the white grubs.

HYPOTHESIS

The differential susceptibility of white grub species to nematodes is both due to the differences in bacterial virulence and nematode's ability to vector the bacteria into the hemolymph.

MATERIALS & METHODS

❖**Symbiotic bacteria isolation** The symbiotic bacteria were isolated from the surface sterilized IJs of *H. bacteriophora* GPS11 or *S. scarabaei* AMK001 by crushing the nematodes and plating on the Brain Heart Infusion medium.

❖**Bacterial pathogenicity** Third instars of white grub species *R. majalis*, *P. japonica*, and *C. borealis* were injected with 2, 20, 200, 2000, or 20000 cells per grub through the proleg into the hemolymph. The grub mortality was recorded at 24 h intervals until 96 h.

❖**Bacterial growth rate *in vitro* and *in vivo*** *In vitro* growth rate was determined by measuring OD600. *In vivo* bacterial growth rate in *R. majalis*:was measured by counting the numbers of colony forming units (CFU) following infection at 24 h intervals.

❖**Nematode pathogenicity** Five IJs in 10µl sterile water were introduced into the gut or hemolymph of third instars *R. majalis*. Orally injected grubs were dissected, and the numbers of nematodes in the gut and hemolymph were counted.

RESULTS

❖**Contribution of symbiotic bacteria to nematode pathogenicity** Both bacteria were virulent to three grub species even at as low as 2 cells per grub (Figs. 2-4)

Pathogenicity of bacteria against *P. japonica*

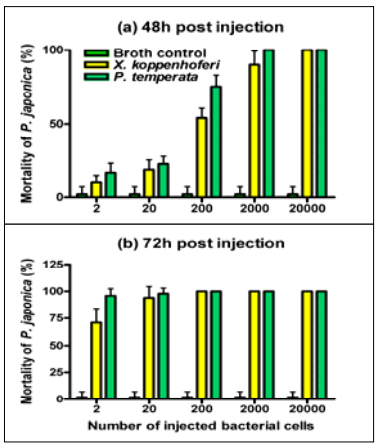


Fig. 2. Mortality of *Popillia japonica* caused by the injection of bacteria or broth (control) into the hemolymph.

Pathogenicity of bacteria against *R. majalis*

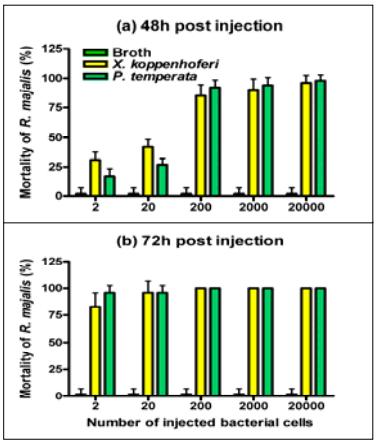


Fig. 3. Mortality of *Rhizotrogus majalis* caused by the injection of bacteria or broth (control) into the hemolymph.

Pathogenicity of bacteria against *C. borealis*

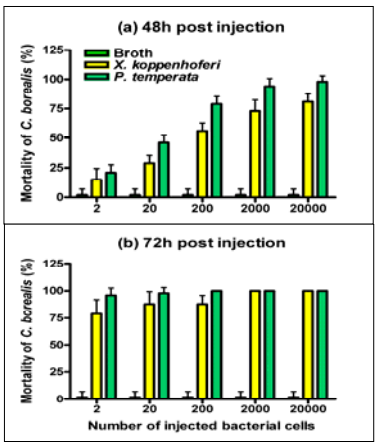


Fig. 4. Mortality of *Cyclocephala borealis* cause by the injection of bacteria or broth (control) into the hemolymph.

❖**Differences in bacterial pathogenicity** *P. temperata* was more virulent than *X. koppenhoferi* to *C. borealis*, but no differences against *P. japonica* and *R. majalis* (Fig. 5).

Bacterial median lethal dose and time

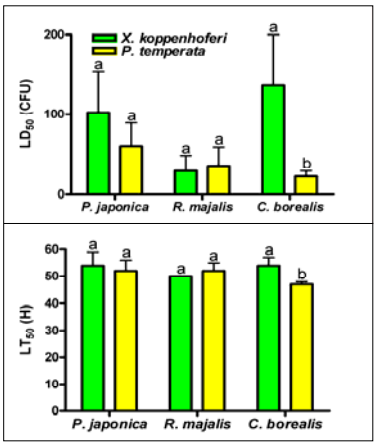


Fig. 5. The median lethal dose LD_{50} at 48 h post injection and median lethal time LT_{50} at 20 cells /grub.

❖**Bacterial growth rate** *P. temperata* grew faster than *X. koppenhoferi* both in vitro and *in vivo* (Fig. 6)

Bacterial growth rate *in vitro* and *in vivo*

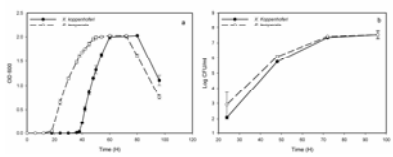


Fig. 6. Growth curves for *X. koppenhoferi* and *P. temperata*. (a) Growth curve in culture derived from measuring OD600 overtime. (b) Growth curve during a grub infection derived from the mean number of CFU recovered from infected grubs at different time points after injection of bacteria.

❖**Nematode pathogenicity** Hemolymph introduced *H. bacteriophora* is virulent to *R. majalis*, but oral introduced *H. bacteriophora* is not (Fig. 7)

Pathogenicity of *H. bacteriophora* to *R. majalis*

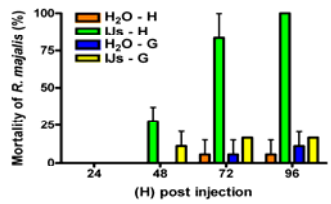


Fig. 7. *H. bacteriophora* pathogenicity when injected into the hemolymph (IJs-H) or gut (IJs-G) of *R. majalis*.

DISCUSSION & CONCLUSIONS

❖ Symbiotic bacteria *P. temperata* and *X. koppenhoferi* are both highly virulent to all three white grub species.

❖ *P. temperata* is more virulent than *X. koppenhoferi* to *C. borealis*, but there are no differences against *P. japonica* and *R. majalis*.

❖ *P. temperata* grows faster than *X. koppenhoferi* both in the media and in the grub *R. majalis* suggesting that the low susceptibility of *R. majalis* to *H. bacteriophora* is not due to the symbiotic bacteria.

❖ Hemolymph introduced *H. bacteriophora* is highly virulent to *R. majalis* suggesting the successful bacterial release by the nematodes if they reach the hemolymph;

❖ Orally introduced *H. bacteriophora* is not virulent to *R. majalis*, and dissections of *R. majalis* confirmed that the nematodes were unable to penetrate into the hemolymph through the gut wall. Therefore, we conclude that the low suscpttibility of *R. majalis* to *H. bacteriophora* is due to nematode's poor ability to penetrate through the gut wall to vector the bacteria into grub's hemolymph.

ACKNOWLEDGMENTS

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